Do Prokaryotes Contain Microtubules?†

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INTRODUCTION

Microtubules comprise a well-defined class of proteinaceous structures composed of tubulin and microtubule-associated proteins found in eukaryotes (29, 78, 91, 127). Three classes of tubulin, α, β, and γ, are known by their amino acid sequences. The evolutionary homology of microtubules is evident from their ultrastructure and biochemical similarity in all eukaryotes. Homologies have been verified by direct and indirect (gene) amino acid sequence analysis in tubulin isolated from over 50 different sources (78, 104). Heterodimers of α- and β-tubulin make up the 13 protofilaments of the tubule walls (Fig. 1), whereas γ-tubulin (104) is associated with centrosomes and other microtubule-organizing centers (MTOCs) (Fig. 2) (118, 119, 138). γ-Tubulin may organize the more abundant α- and β-tubulins that form the tubule walls (103). Thus, the term "microtubule" has a highly specific meaning for those who study eukaryotes, whereas bacteriologists and biochemists (those primarily concerned with prokaryotes) tend to use the term far more generally to refer to any of a large number of fibrous hollow cell structures. Here we refer to prokaryotic thin tubes as cytoplasmic tubules to distinguish them from bonafide microtubules of eukaryotes. Unless biochemical and sequence characterization warrants, we advocate avoidance of the term microtubule for the cytoplasmic tubules of prokaryotes.

Cytoplasmic tubules of various diameters, lengths, and descriptions have been labeled microtubules in electron micrographs of bacteria. However, unlike the microtubules of eukaryotic cells, the biochemical composition of bacterial tubules is largely undetermined and no functions have been ascribed to them. The presence of tubulin-based microtubules in prokaryotes would have implications for theories of cell evolution (12, 42, 58, 68, 83, 84, 110, 120). This review assembles published information on such structures in prokaryotes and addresses whether any prokaryote cells contain tubulin-based microtubules.

MICROTUBULES IN EUKARYOTIC CELLS

Typical eukaryotic microtubules have an outer diameter of approximately 24 nm and an inner diameter of approximately 14 nm (6, 65) and are variable in length (ranging from a few tens of nanometers to over 3 mm in ctenophore comb cilia). In the sperm of plants (e.g., ferns, mosses, ginkgos) and those of most animals, the sperm tail and the cilia are composed of microtubules arranged in the well-known nine-doublet-and-two-singlet-tubule array. The shaft, always intracellular, which displays this [9(2)+2] pattern in transverse section, as determined by thin-section electron microscopy, is called the axoneme. Generally composed of 13 protofilaments, each 5 nm in diameter (Fig. 1), microtubules from different sources are made of 12 (43) to 15 (99) protofilaments. The molecular mass of each protein subunit, α- and β-tubulin, is approximately 50 kDa. When analyzed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis, the apparent molecular mass of the two subunits ranges from 53 to 57 kDa depending on the SDS and buffer system used (36). The tubulin protein molecule composing the protofilaments is a heterodimer with an apparent molecular mass of 110 to 120 kDa. Amino acid sequencing of α- and β-tubulins indicates that they are among the most highly conserved proteins known (76–78); γ-tubulin differs from both α and β about equally (104). The similarity between the α subunits of chicken and human tubulin is 95%, while the

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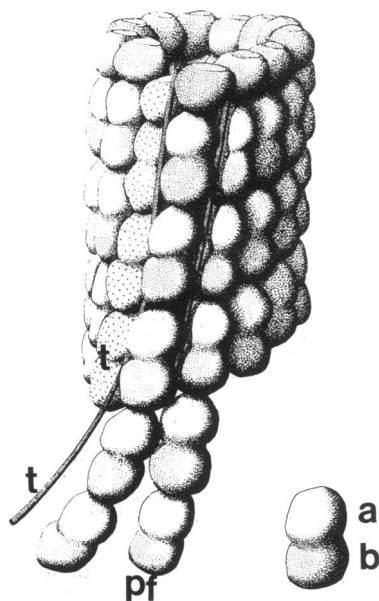
FIG. 1. Tectin-containing axonemal microtubule. Abbreviations: pf, protofilament; a and b, α and β heterodimeric tubulin protein; t, tectin showing two possible relations of tectin to tubulin. The inner diameter is 14 nm, and the outer diameter is 24 nm. (Drawing by Christie Lyons.)

similarity of α- and β-tubulin, in either of the species, is around 50% (76–78). γ-Tubulin, by contrast, has only 37% sequence homology with α- and β-tubulin (104). Apparently γ-tubulin is not in the walls of microtubules but, rather, is associated with MTOCs such as centrosomes (118, 119, 138). Sequence conservation is so marked that a newly isolated protein thought to be tubulin would have to share sequence homology with α-, β-, or γ-tubulin.

Assays used to indicate the presence of tubulin (96) include reactions with monoclonal or polyclonal anti-tubulin antibodies, reactions with specific tubulin-binding drugs (taxol, colchicine, vinblastine, colcemid, and related alkaloids; compounds such as podophyllotoxin [131] and its derivative β-peltatin [132]; and many others [18, 28]). Since in vitro soluble tubulin assembles into microtubules, ultrastructural and various viscometric techniques are also used for tubulin assay (29).

Microtubules are nearly universally distributed in eukaryotes. They are reported to be absent only in certain algae (e.g., Nanochlorum species [137]). This apparent absence may reflect secondary loss. The near ubiquity of eukaryotic microtubule-based mitotic cell division and axonemal motility, as well as the identity of kinetosomes and centrioles composed of nine triplet microtubules and lacking central ones [9(3)+0], lends support to the concept that [9(2)+2] axonemes and mitotic spindles are related and appeared early in the evolution of eukaryotes (12, 20). Other microtubular functions, such as cytoplasmic transport (5), may have evolved before these complex structures.

Bacterial flagella, structures that rotate because of "motors" in their bases, entirely lack microtubules. The analogous but clearly nonhomologous structures of eukaryotes that contain 24-nm microtubules in the nine pairs of doublets with shared walls that surround two central singlet tubules (i.e., axonemes) include all cilia and eukaryotic flagella, parts of the photoreceptor cells of the eye, the axoneme of the undulating membrane of trypanosomatids, and many other structures for which

the general name "undulipodia" has been used (82–85, 88, 89, 115). Axonemal outer doublets have tectin proteins (three different molecular masses are reported in sea urchin sperm axonemes) that extend down their lengths (74). Tectin, which is far less abundant per axoneme than is tubulin, may make up one of the protofilaments or may form an additional structure that fits between the tubulin protofilaments. Both alternatives are shown in Fig. 1.

CYTOPLASMIC TUBULES OF PROKARYOTIC CELLS

Efforts seeking tubulin in bacteria have so far been unsuccessful (14, 78, 98). However, the *ftsZ* (filament temperature sensitivity Z) gene in *Escherichia coli* and its gene product with a 7-amino-acid tubulin motif (15) warrant careful comparison with eukaryotic tubulins (described below).

Many cytoplasmic tubules and fibrous structures within the size range of tubulin tubules and tubulin protofilaments exist in prokaryotes (Table 1). Few biochemical or molecular sequence data are available on their composition; therefore, their presence is determined primarily by morphology. In our tabulation of cytoplasmic structures, descriptions of rhapidosomes (from the Greek for **rhapis** [rod] and **soma** [body]) (3, 72, 73, 109, 135), probably defective phage tails (26), and all extracellular structures have been excluded. Many reports of tubules in prokaryotes may be attributable to viral infection. We also omit membranous structures (e.g., mesosomes) and cases for which we judge that fixation or partial fixation caused tubular structural artifacts.

Eubacteria

γ subdivision of the purple bacteria. (i) Azotobacters. Microtubule-like structures have been found in the nitrogen-fixing bacterium *Azotobacter agilis* (113). Cytoplasmic tubules composed of 12 protofilaments were observed in negatively stained and thin-sectioned material of *A. vinelandii* 0 (111, 126). They are oriented longitudinally and are more prevalent in dividing cells. Extracts of *A. vinelandii* gave a weak positive reaction to anti-tubulin antibodies (1). A partial purification of these tubules on sucrose density gradients and electrophoretic separation yielded three polypeptides with molecular masses from 45 to 66 kDa. *A. vinelandii* genomic DNA was probed with a plasmid containing a *Drosophila* tubulin gene (2). Although the data implied the presence of a tubulin homolog in *Azotobacter* species, this work was never pursued. Electron micrographs of the cytoplasmic tubules, showing their substructure and orientation, are presented in Fig. 3.

(ii) Enteric bacteria. Studies of *E. coli* cell division have revealed proteins possibly ancestral to tubulins (15). The *ftsZ* gene encodes a protein that multimerizes to form a ring in the early stages of cell division. Like tubulin, FtsZ protein responds to GTP (25, 97, 112): membrane-associated FtsZ protein binds and hydrolyzes GTP. Thirteen amino acid residues are entirely conserved (in *E. coli*, *Bacillus subtilis*, and *Rhizobium meliloti*), and seven of them (Gly Gly Gly Tyr Gly Ser Gly) are considered to form a "tubulin signature sequence" (9, 25). This motif may be related to GTPase activity in multimerization steps that lead to the formation of the FtsZ ring (25). The FtsZ ring is a dynamic structure which apparently contracts during cell division, probably by self assembly and disassembly on the cytoplasmic surface of the membrane at the position where septation will occur (15). A better understanding of the relation of the *ftsZ* gene product to tubulin, if any, must be discerned before any evolutionary homology can be posited. Because relatively little is known
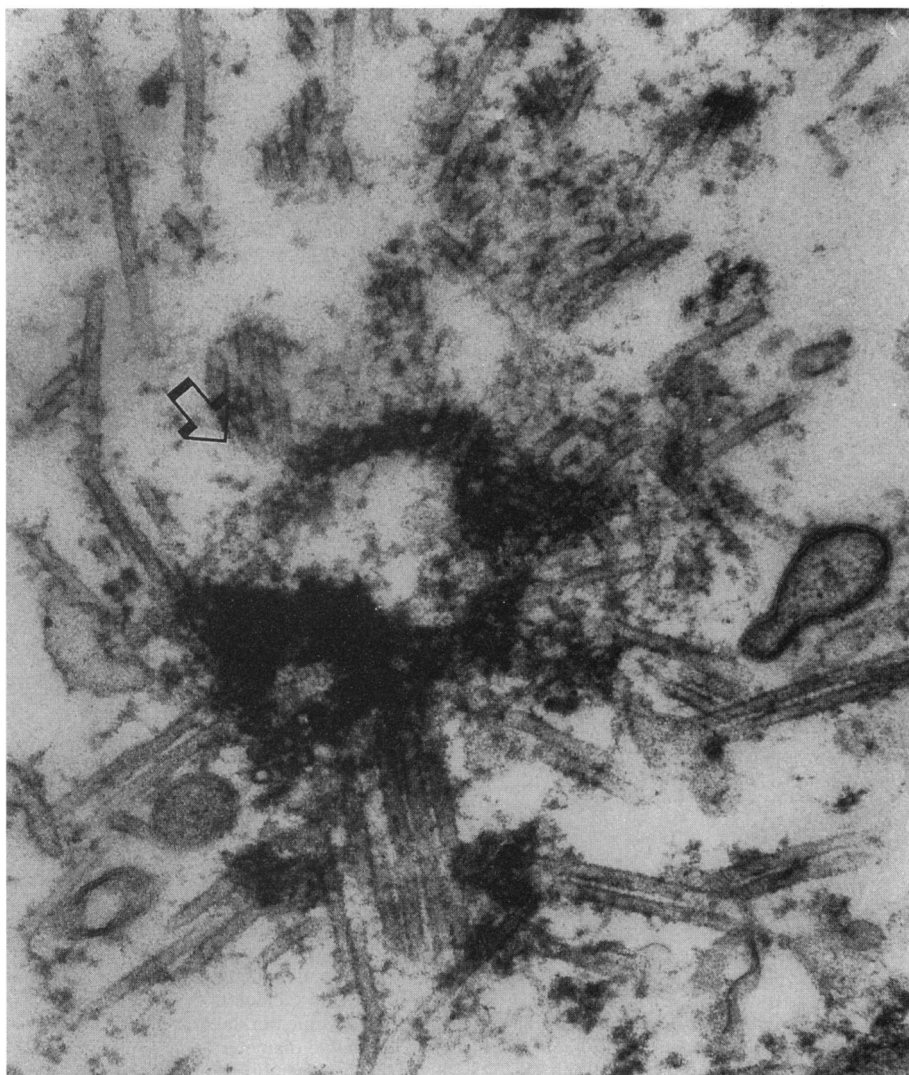
FIG. 2. MTOC (arrow) in a nassulid ciliate. Courtesy of J. B. Tucker.

about GTP-binding proteins in bacteria, the apparent related-ness of tubulin to FtsZ may be superficial. The discovery of *ftsZ* may stimulate further surveys for homologs of eukaryotic tubulin through the use of PCR and other techniques. FtsZ is not related to the *Rhizobium* ORF2 protein (94), which has been reported to have minor similarities to α-tubulin. In *E. coli*, large tubes (30) may be membrane vesicles associated with excessive fumarate reductase synthesis (32).

Tubules from *Proteus mirabilis* are typical of those associated with viral infections (124, 125). Treatment of cell cultures with mitomycin, known to induce lysogenic phages, induced struc-tures resembling polymerized T4 phage tail sheaths (66). Cytoplasmic tubules were reported to occur in enteric bacteria including the marine bacteria *Vibrio psychroerythrus* (24) and *Vibrio marinus* (33). The *V. psychroerythrus* tubules cross the septum of dividing cells (24).

δ subdivision of the purple bacteria. Cytoplasmic tubules were reported to occur in *Chondromyces crocatus* (79). How-ever, these structures may be associated with rhapidosomes, which have sometimes been observed in these bacteria. Tubu-lar structures were also reported to occur in *Myxococcus*

xanthus (19). These bacteria also possess cytoplasmic fibers with periodic striations.

Cyanobacteria. Cytoplasmic tubules have been reported to occur in numerous cyanobacteria (56–58). Some occur in specific cell types, e.g., the small cells of motile hormogonia (57, 61), and may play a critical role in the function of these cells. Cylindrical bundles of tubules were seen in *Synechococ-cus* sp. (8), and an elaborate plate-shaped tubular complex associated with the cell wall was observed in *Nostoc* sp. strain 756 (17). These tubules, arranged in a hexagonal array, appar-ently break into smaller linear structures which were referred to as protofilaments. This *Nostoc* cell wall complex was re-ferred to as a primitive type of MTOC or centriole equivalent (17). A similar plate-tubule array was observed in *Anabaena* sp. strain B-378 (59), in which long tubules were associated with the plate. Small filaments (referred to as microfilaments) were observed in bundles in at least three *Anabaena cylindrica* strains (B-629, Wolk, and B-381) and in *Anabaena* sp. strain B-380 (60). Treatment of cells with cytochalasin B, which often inhibits actin fibrils and their activities, did not disrupt the prokaryotic filaments. A nearly identical structure to that

TABLE 1. Cytoplasmic tubules, fibers, and proteins possibly related to tubulin found in prokaryotes

Classification[a]	Organism	Cytoplasmic structures[b]		Comments	Reference(s)
		Tubules	Fibers		
I. Eubacteria					
A. γ subdivision of the purple bacteria (Fig. 3)	*Azotobacter agilis*	+		Structures seen in vesicles produced by osmotic shock	113
	Azotobacter vinelandii	19–24		Tubules with 12 protofilaments, weak anti-tubulin antibody and Southern blot positive; tubules are longitudinally oriented and more prevalent in dividing cells	1, 2, 111, 126
	Escherichia coli	60–85		Tubules probably composed of membrane containing fumarate reductase; FtsZ may be homologous to tubulin	15, 25, 30, 32, 97, 112
	Proteus mirabilis	20		May be phage associated	66, 124, 125
	Vibrio marinus	21		May be membranous or phage associated	33
	Vibrio psychroerythrus	10–15		Cross the septum of dividing cells	24
B. δ subdivision of the purple bacteria	*Chondromyces crocatus*	+		Probably phage associated	79
	Myxococcus xanthus	+	4–5		19
C. Cyanobacteria (Fig. 4)	*Anabaena* sp. strains B-380 and B-381	+	2.8	Cytochalasin B and colchicine insensitive	56, 61
	Anabaena sp. strain B-378	10	2.8	Associated with "plate" structures (Fig. 4)	56, 59, 61, 62
	Anabaena sp. strain 1448	+		Associated with polyhedral bodies	56, 61
	Anabaena aequalis	+		Associated with polyhedral bodies	56, 61
	Anabaena catenula	+		Associated with polyhedral bodies	56, 61
	Anabaena cylindrica B-629, B381, and Wolk	+	2.8	Cytochalasin B insensitive, polyhedral body-associated tubules	56, 60, 61
	Anabaena minutissima	18–23		Striations with a 20 to 25 periodicity	56, 57, 60, 61
	Anabaena variabilis	+		Associated with polyhedral bodies	56, 61
	Calothrix sp. strain UTEX B1827	10–14, 18–22		Plasma membrane associated; multiple sizes	34, 56
	Calothrix sp. strain Cambridge 1410/6	+		Microplate-tubule array	34, 56
	Calothrix anomala	+	+	Plasma membrane associated; multiple sizes	34, 56
	Calothrix brevissima	+	+	Plasma membrane associated	34, 56
	Calothrix javanica	+		Plasma membrane associated; multiple sizes	34, 56
	Calothrix marchica	18–23		Periodic striations	56, 57, 69
	Calothrix membranacea	+		Plasma membrane associated; multiple sizes	34, 56
	Calothrix parientina	+		Variable diameters	34, 56
	Calothrix pulvinata	10–14		Plasma membrane associated	34, 56
	Calothrix scopulorium	+	+	Variable diameters	34, 56
	Fremyella diplosiphon	10–14, 18–22	+	Plasma membrane associated; multiple sizes	34, 56
	Gloeotrichia sp. strain UTEX 941	18–22	+	Plasma membrane associated	34, 56
	Nostoc sp. strains 387 and 389	10–15		Microplate-tubule array; variable diameters; only in actively growing cultures	56, 61, 62
	Nostoc sp. strain 756	17.5	3.5–5.0	Associated with a "plate" structure at a 90° angle (Fig. 4)	17, 56, 61, 62
	Nostoc ellipsosporum B1623	+		Variable diameters	56, 67, 61
	Nostoc muscorum 1545	23		Periodic striations	56, 57
	Nostoc punctiforme 1629	+		Variable diameters	56, 57, 61
	Nostoc sphaericum	17.5		Associated with a plate structure at a 90° angle (Fig. 4); colchicine insensitive	56, 57, 61
	Nostoc zetterstedtii	+		Variable diameters	56, 57, 61
	Schizothrix calcicola	14		Associated with an arched "plate"	13, 56, 67
	Synechococcus sp.	15		Occur in bundles	8, 34, 56, 67
D. Spirochetes (Fig. 5 and 6)	*Diplocalyx calotermitidis*	24			10, 38
	Hollandina sp.	21		Anti-tubulin positive, probably as a result of hsp65 stress protein	10, 90, 98
	Leptospira illini	7			50
	Leptospira icterohaemorrhagiae	+			136
	Pillotina sp.	21		Anti-tubulin positive, probably as a result of hsp65 stress protein	10, 44, 90, 98
	Spirochaeta bajacaliforniensis	–	+	Anti-tubulin positive, probably as a result of hsp65 stress protein	11, 35, 98
	Treponema calligyrum	7		Probably similar to other treponemes, e.g., *T. pallidum*	47, 49

TABLE 1—*Continued*

Classification[a]	Organism	Cytoplasmic structures[b]		Comments	Reference(s)
		Tubules	Fibers		
	Treponema microdentium	7		Probably similar to other treponemes, e.g., *T. pallidum*	47, 49
	Treponema minutum	7		Probably similar to other treponemes, e.g., *T. pallidum*	47, 49
	Treponema pallidum	7		Cytoplasmic structures apposed to the region of the periplasmic flagella	45, 70, 105–108, 129
	Treponema pertenue	7		Probably similar to other treponemes, e.g., *T. pallidum*	49, 54
	Treponema phagedenis	7		Probably similar to other treponemes, e.g., *T. pallidum*	46, 49
	Treponema refringens	8–12	+	97-kDa protein	31, 46
	Treponema reiteri	7.5		6–8 tubules; sometimes associated with the base of the rotary motor	49, 52
	Treponema vincentii	7		Probably similar to other treponemes, e.g., *T. pallidum*	46, 49
E. Unidentified; probably gram negative	'Skinny gliders'			Anti-tubulin positive, probably as a result of hsp65 stress protein	90, 98
	Cockroach symbionts	+		Tubules traverse the septum of dividing cells	37
F. Gram-positive bacteria (Fig. 7)	*Acholeplasma laidlawii*	48		Anti-tubulin negative; does not copolymerize with tubulin; resembles viral coat protein	14, 75, 92
	Arthromitus sp.		+	Spore attachment filament	88
	Group D streptococcal L-form	25		May be wall material or membrane	22, 23
	Frankia sp.	45	6.5	Longitudinally oriented	71
	Mycoplasma pneumoniae	54		Structure described as a "core"	93, 95, 128, 133
	Mycoplasma rho form	–	+	65-kDa protein; 26-kDa protein reassembles tubulin in its response to temperature and ionic concentration	114
	Mycoplasma gallisepticum	–	–	Tubulin-like protein	101, 102
	Spiroplasma citri		4	39-kDa protein	21, 121, 122
II. Archaebacteria	*Halobacterium halobium*	–		55-kDa protein reacts with anti-tubulin antibodies; DNA reacts by Southern blot	117

[a] Classification based on that of Woese (134).
[b] Outer diameter (nanometers) is given if known. +, present but not measured; –, not detected.

described by Jensen and Ayala (59), with an arched plate and projections of tubules, was reported to occur in *Schizothrix calcicola* cells (13). A similar structure was also seen in 1% of *Calothrix* spp. (34). Electron micrographs of these cyanobacterial structures are shown in Fig. 4.

Plasma membrane-associated tubules varying in size were observed in *Calothrix anomala, C. brevissima, C. javanica, C. membranacea, C. pulvinata,* and *Fremyella diplosiphon* (34). Tubules of similar diameter, insensitive to colchicine treatment, were also observed in *Anabaena* sp. strain B-378, *Nostoc zetterstedtii, N. ellipsosporum* B-1623, *N. punctiforme* 1629, *Nostoc* sp. strain 380, and *Nostoc* sp. strain 387 (61). Tubules oriented perpendicularly to the plasma membrane were occasionally found in actively growing *Nostoc* cultures: *N. ellipsosporum* B-383 and *N. punctiforme* 1629 (61).

Tubules were seen throughout the cytoplasm of *Anabaena minutissima* B-1613 (60, 61). These tubules had fine striations perpendicular to the longitudinal cell axis. Other tubular structures were observed in *Calothrix marchica* (69), *Calothrix* sp., *C. javanica, C. membranacea, C. anomala, C. parientina, C. scopulorum, Fremyella diplosiphon,* and *Gloeotrichia* sp. (34). They varied in diameter and occurred singly or in groups of five or more.

Spirochetes. Cytoplasmic tubules and fibers have been seen in the protoplasmic cylinders of many spirochetes (Fig. 5 and 6). They were discovered in large spirochetes symbiotic in termite hindguts by Hollande and Gharagozlou (44). Tubules

with a 24-nm outer diameter were seen in the spirochete *Diplocalyx calotermitidis* (38) (in the family *Pillotinaceae* [87]). Similar cytoplasmic tubule structures were observed in two other genera of this family, *Hollandina* and *Pillotina* (10, 90) and in a thinner *Diplocalyx* species from *Cryptotermes cavifrons* (Fig. 6C) (7). The cytoplasmic spheres from which the tubules emanate are reminiscent of fungal and ciliate MTOCs (41, 123) (Fig. 2). Up to three such MTOC-like structures per cell were observed in *Diplocalyx* species isolated from termites in Mississippi and Florida (Fig. 6C). Smaller tubules (7 nm) in *Treponema* and *Leptospira* (*Leptonema*) spirochetes were reported by Ovčinnikov, Hovind-Hougen, and others (16, 45–47, 49, 50, 52, 54, 55, 70, 105–108, 136). Bundles of six to eight cytoplasmic tubules apparently emanate from the proximal face of the flagellar basal discs (49) (Fig. 5A). They extend through the cytoplasm to overlap with a similar bundle of cytoplasmic tubules that extends from the other terminus in *Treponema reiteri* (49, 52). The tubules lie close to the inner layer of the plasma membrane underlying the region occupied by periplasmic flagella (129). Similar tubules were found in the cytoplasm of the spirochetes *Treponema pallidum, T. phagedenis, T. vincentii, T. refringens, T. calligyrum, T. minutum, T. microdentium, T. pertenue, T. caniculi,* and *Leptospira illini* (45–47, 52, 54, 55, 70, 105–108, 129) but were absent in *Treponema genitalis* (27, 48, 49). The presence of such small cytoplasmic tubules is a taxonomic characteristic of the genus *Treponema* (53). Tubules or fibers are also seen in the modified
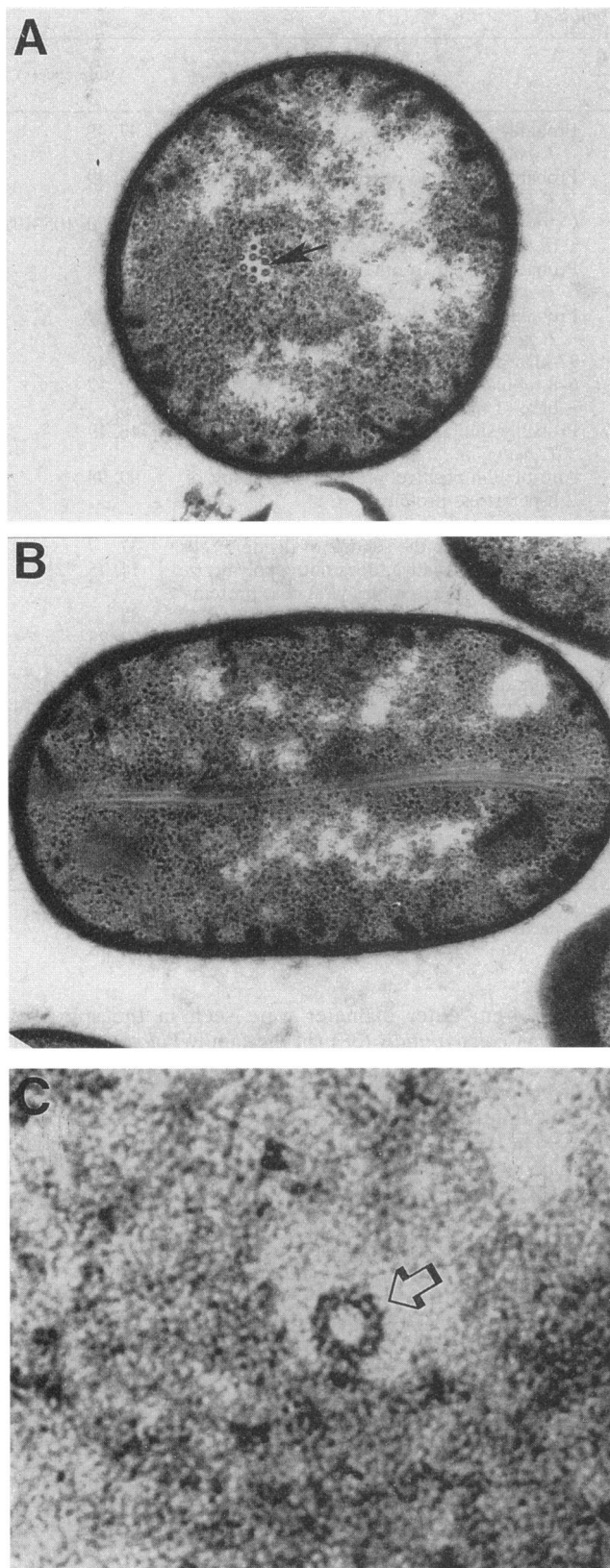
FIG. 3. Cytoplasmic tubules in *Azotobacter vinelandii*. (A) Transverse thin section of eight or more tubules (arrow). Magnification, ×52,000. (B) Longitudinal section with tubules that span most of the cell. Magnification, ×46,000. (C) One of the tubules shown at high magnification (arrow) suggests that they are composed of 12 protofilaments. Magnification, ×512,000. Courtesy of Ross Payne and Michael Adams.

ends (attachment sites) of small spirochetes isolated from termite hindguts (Fig. 5C).

Aerobic or microaerophilic spirochetes with "bent ends" formerly all classified as *Leptospira* are now divided into two genera: *Leptonema*, with cytoplasmic tubules and gram-positive-like cell walls, and *Leptospira*, which lacks tubules and has the typical gram-negative cell wall (50). Similarly, the genus *Brachyspira* (53) was established to accommodate treponeme-like spirochetes that lack these tubules. Spirochetes of the genus *Borrelia* also lack tubules (51). Twelve spirochete genera were compared with respect to the presence (*Hollandina*, *Diplocalyx*, *Leptonema*, *Pillotina*, and *Treponema*) and absence of tubules (86).

Tubules were isolated from sonicated cells of *Treponema refringens* and observed by transmission electron microscopy after negative staining (31). Two tubular or fibrillar types can be distinguished: wide fibrils (12 nm) with periodic striations, and narrow fibrils (8 nm) without striations. In addition, a smaller ribbon structure (5 nm) runs apposed to the fibrils. The fibrillar fraction contains a major polypeptide with an apparent molecular mass of 97 kDa.

Spirochaeta bajacaliforniensis contains cytoplasmic fibers but no tubules (35). Whole-cell spirochete extracts yielded two copurifying proteins (65 and 45 kDa) in the same warm-cold cycling procedure that is routinely used to enrich eukaryotic tubulins (11). Soluble fractions of these proteins (called S1 and S2, respectively) form fibers when warmed to 37°C, similar to tubulin. Immunoblots with anti-tubulin antibodies demonstrated reactivity with S1 protein. This S1 protein was also detected by antigenic activity in immunoblots of *Spirochaeta* sp. strain BA-4, *S. litoralis*, *S. halophila* (11), and *S. isovalerica* (97a). The S1 protein has now been identified as homologous to the hsp65 family (98), including the *E. coli* GroEL, X-GroEL from bacterial symbionts of *Amoeba* species (4), and the mycobacterium common antigen protein (116). As a result of temperature-dependent precipitation, preparations of tubulin used as antigen may be contaminated with similar proteins, or such proteins may be present in the adjuvant. Three synthetic oligonucleotide probes with sequences similar to conserved regions of the α- and β-tubulin genes failed to hybridize with genomic DNA in any spirochete or other prokaryote tested (97a). There are many observations of cytoplasmic tubules in certain spirochete genera (Table 1; Fig. 5 and 6) and evidence for their absence in others (40, 49–51, 53), yet evidence for tubulin at either the gene or protein level is entirely lacking. The S2 protein (11), which did not react with anti-tubulin antibodies, is similar in molecular weight to the *E. coli* FtsZ protein and may warrant further investigation.

Gram-positive bacteria. (i) Mycoplasmas. Some mycoplasmas, eubacterial prokaryotes that are related to gram-positive bacteria and lack cell walls, contain fibrous structures in their cytoplasm (21, 81, 92, 93, 95, 100–102, 114, 121, 122, 130, 133). The fibers tend to be smaller than microtubules and more similar to actin (80). Such fibers (composed of a 26-kDa protein), termed a rho body in *Mycoplasma mycoides*, form a striated structure that extends axially through the cell and terminates at the plasma membrane (114). A structure referred to as a core has been observed by freeze-fracture in the specialized tip structure of *Mycoplasma pneumoniae* (93, 95,
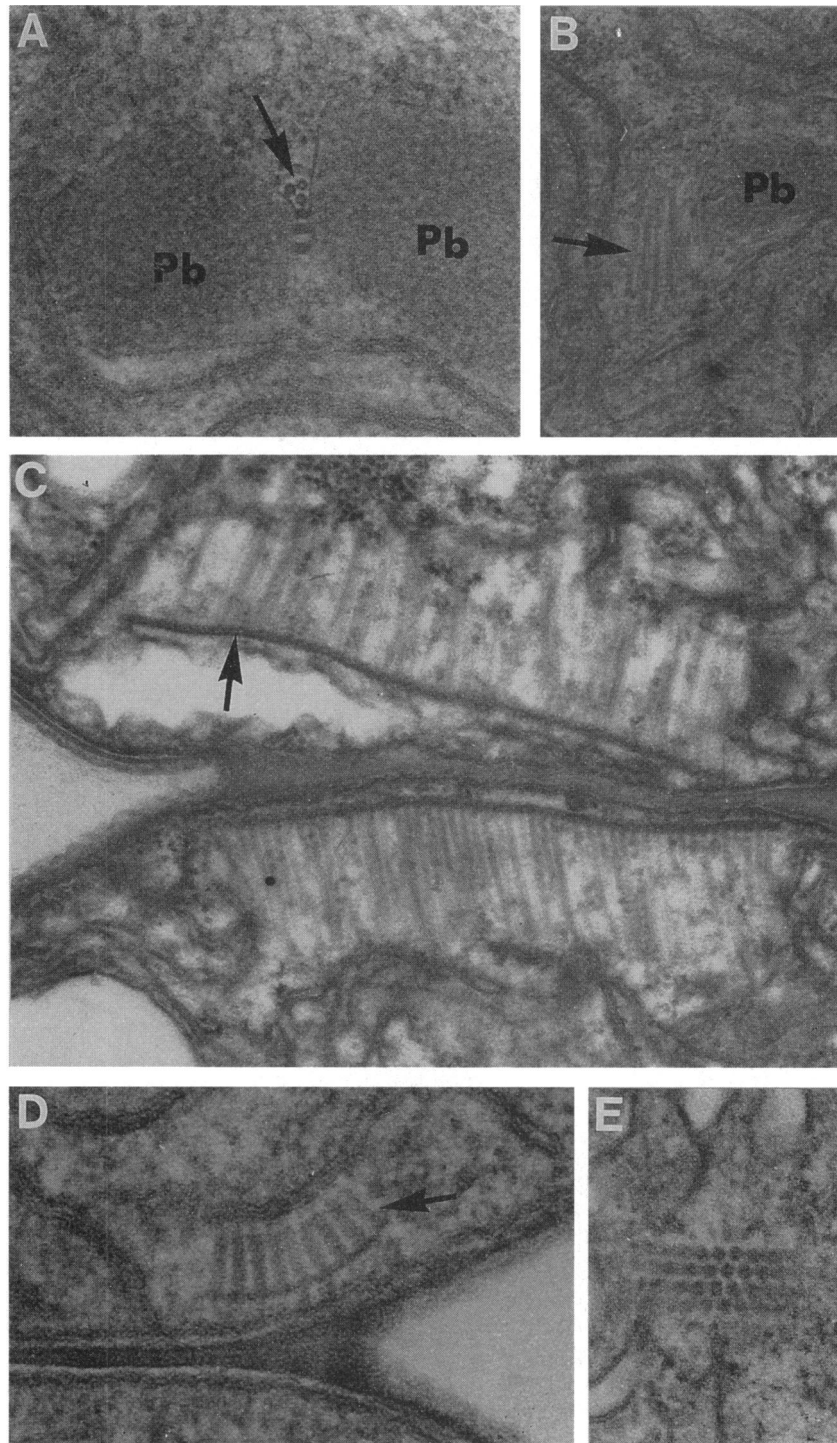
FIG. 4. Cytoplasmic tubules in cyanobacteria. (A) *Anabaena* sp. strain UTEX 1448 with polyhedral body (Pb)-associated cytoplasmic tubules (arrow). Magnification, ×185,000. (B) Longitudinal section of polyhedral body-associated tubules (arrow) in *Anabaena* sp. strain UTEX 1448. Magnification, ×75,000. (C) Plate filament array (arrow) in *Nostoc pruniforme* UTEX LB 756. Magnification, ×150,000. (D) Microplate-tubule array (arrow) in *Anabaena* sp. strain UTEX B378. Magnification, ×150,000. (E) Transverse section of tubules in the microplate-tubule array in *Anabaena* sp. strain UTEX B378. Magnification, ×100,000. Courtesy of Thomas E. Jensen. Reprinted from reference 57 with permission of the publisher.

128, 133). Isolated fibers from the rho body disassemble and reassemble with varying ionic strength and temperature, a property shared with tubulin. Spiral mycoplasmas (motile, wall-less cells that also lack flagella) of the genus *Spiroplasma* possess small (4-nm) cytoplasmic fibers (21, 122, 130) composed primarily of a single 39-kDa polypeptide (121). Complexes of these fibers may be associated with spiroplasma motility (80, 81). Evidence is lacking for homology between
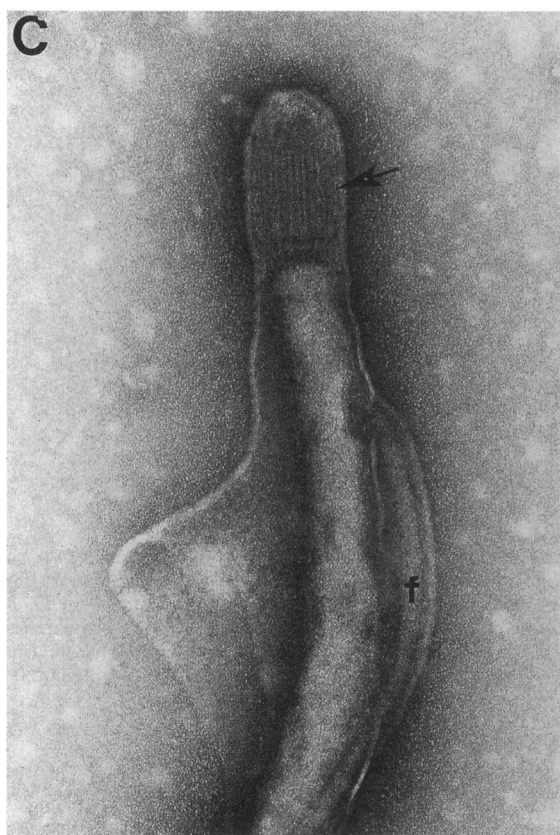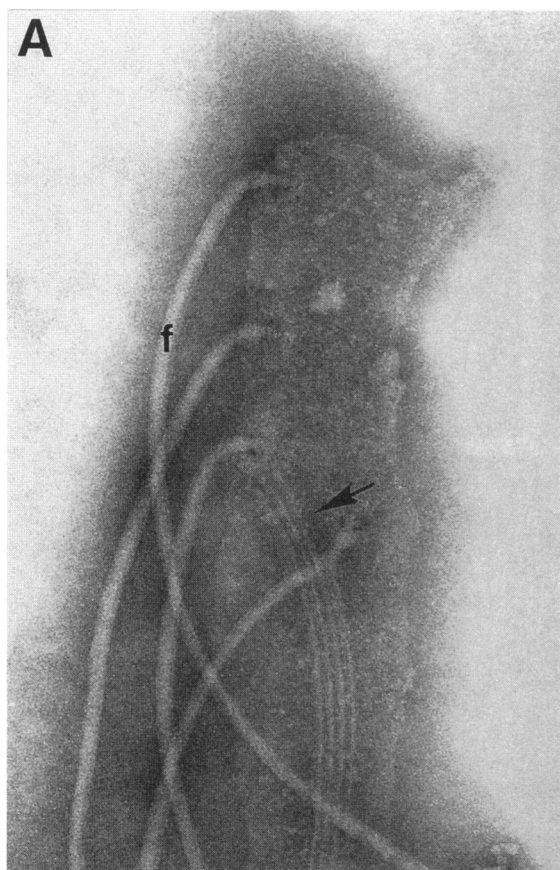
FIG. 5. Cytoplasmic tubules of small spirochetes, negative stain preparations. (A) Cytoplasmic tubules (arrow) of *Treponema reiteri* extend proximally from the rotary motor of the flagellum (f). Magnification, ×112,000. Courtesy of Kari Hovind-Hougen. Reprinted from reference 49 with permission of the publisher. (B) Cytoplasmic fiber (arrow) and flagella (f) in *Spirochaeta bajacaliforniensis* (9a). (C) Modified apex of a termite spirochete with tubular or filamentous structures (arrow), flagellum (f) inserted subterminally. Courtesy of David G. Chase. Reprinted from reference 84 with permission of the publisher.

these fibers and any eukaryotic motility proteins (e.g., actin, myosin, or tubulin) (121).

The mycoplasma *Acholeplasma laidlawii* contains tubule structures larger than eukaryotic microtubules (92). Cell lysates contain smaller (14-nm) helical fibers (67). The major component in the fibers is a 100-kDa protein. Attempts to

copolymerize *Acholeplasma laidlawii* proteins with brain tubulin and to find immunological cross-reactivity with tubulin were unsuccessful (14). Aberrant assemblages of coat protein of an *Acholeplasma* virus resemble these fibrous structures (75).

The possibility that cytoskeletal tubulin-like protein is present in an extensive submembranous tubular network in
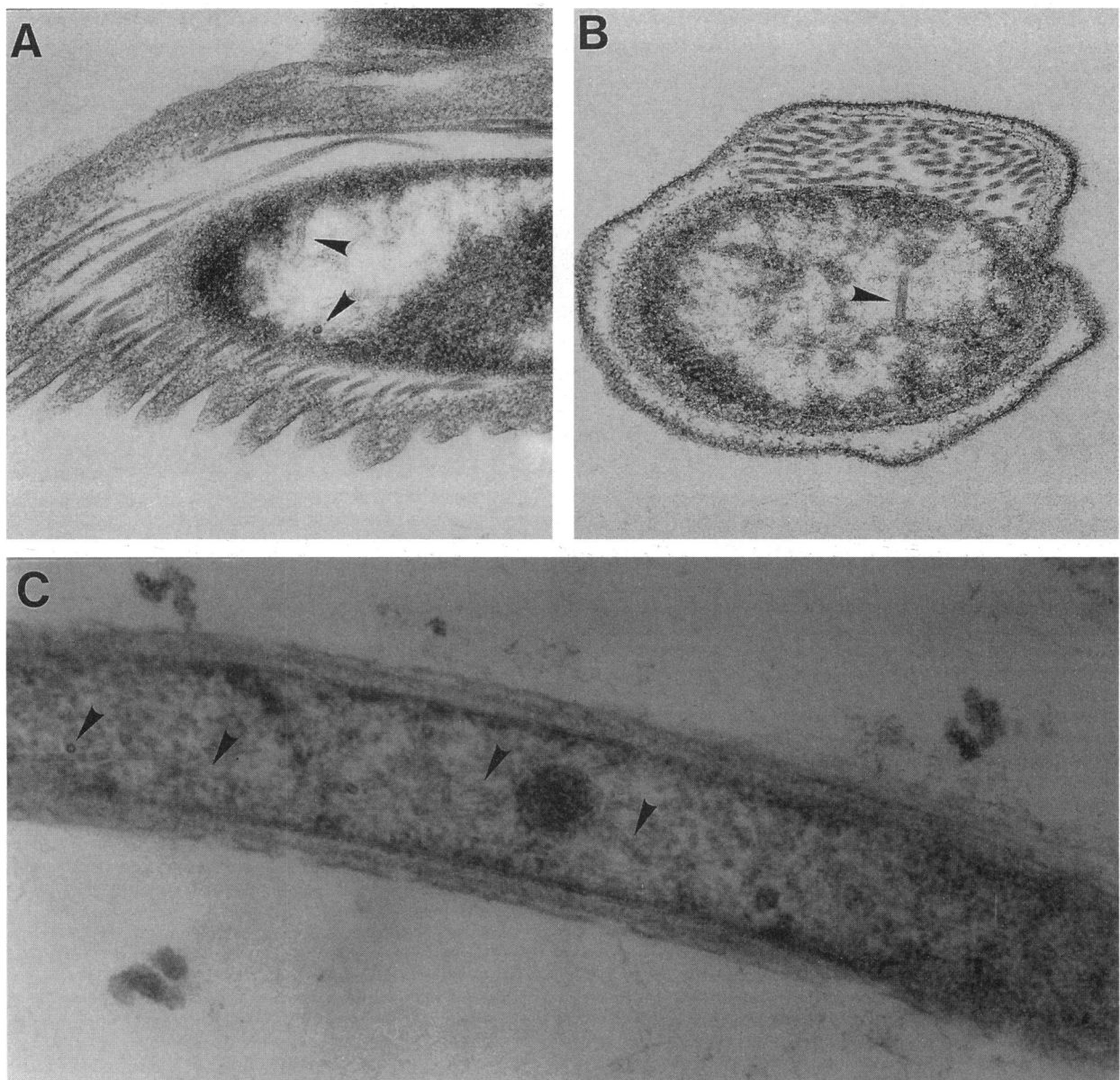

FIG. 6. Cytoplasmic tubules of larger spirochetes symbiotic in termites. (A) Tubular structures (18 nm in diameter; arrowheads) of a *Pillotina* sp. from *Reticulitermes hesperus*. Courtesy of David G. Chase. (B) Tubules (arrowhead) in a *Diplocalyx* sp. from *Incisitermes minor*. Courtesy of David G. Chase. (C) Cytoplasmic tubules (arrowheads) emanating from a center in a *Diplocalyx* sp. from *Cryptotermes cavifrons*. Courtesy of J. B. Ashen. Reprinted from reference 7 with permission of the publisher.
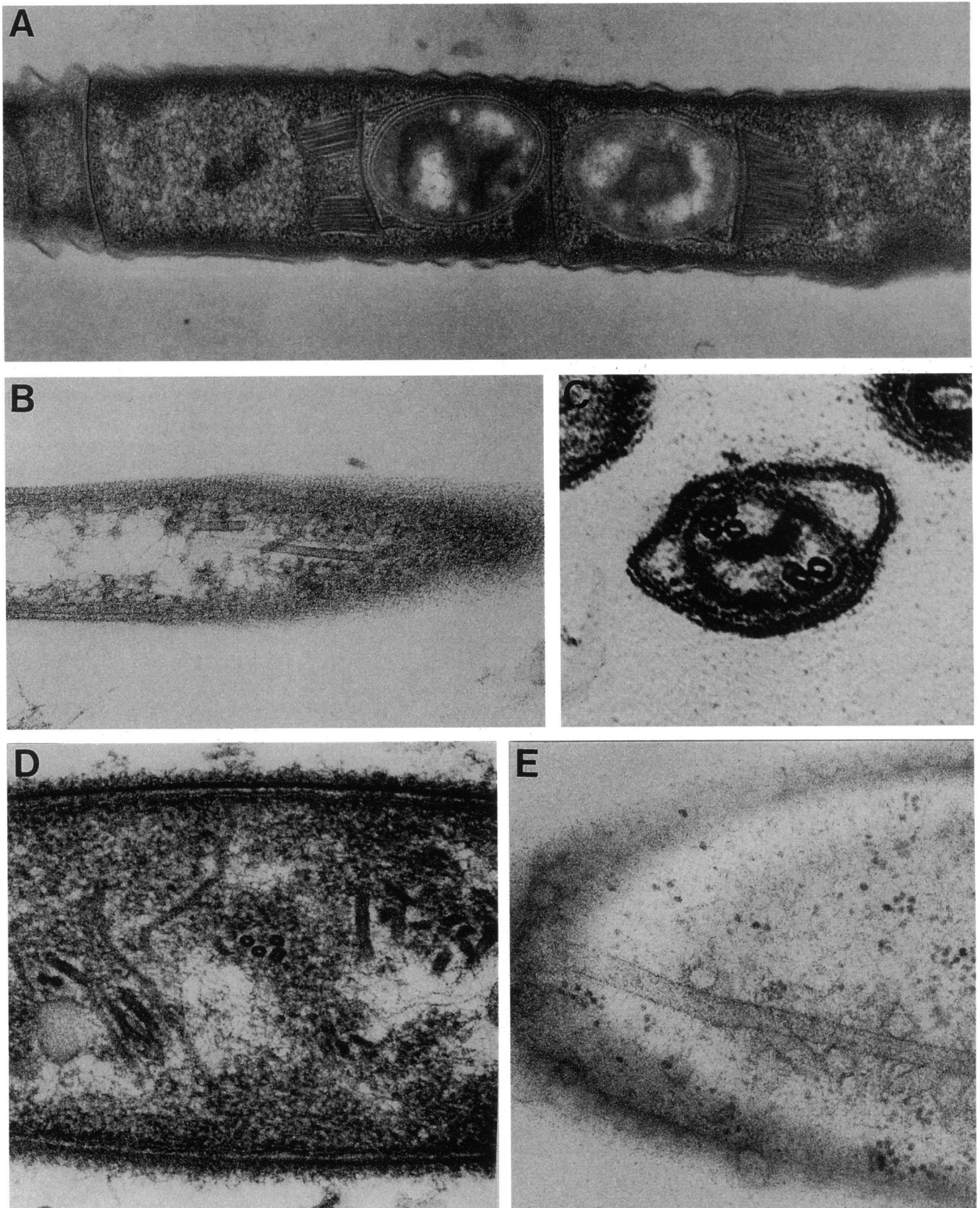
FIG. 7. Tubules in other bacterial taxa. (A) *Arthromitus* endospores with spore appendages. Courtesy of David G. Chase. (B) Cytoplasmic tubules in unidentified bacterium from *Incisitermes minor*. Courtesy of David G. Chase. (C) Transverse section of cytoplasmic tubular pairs in a rod-shaped bacterium from *Incisitermes schwartzi* (12a). (D) Clusters of tubules in an unidentified bacterial symbiont of *Incisitermes minor*. Courtesy of David G. Chase. (E) Cytoplasmic tubule in the actinobacterium *Frankia* sp., symbiotic with *Casuarina* roots. Magnification, ×86,000. Courtesy of Susan Lancelle and Peter K. Hepler. Panel E reprinted from reference 71 with permission of the publisher.

several mycoplasms has been argued by Nikonov et al. (101, 102). This work suggests that a 40-kDa protein associates with a 70-kDa subsurface component in *Mycoplasma gallisepticum* S6, R, 5969, and MRJ, especially at the blebs thought to be related to cell division (39, 101, 102). Nikonov's group also claims that the 40-kDa protein can be phosphorylated in vitro and that these tubules may be the same as the "helical ribosome structures" (39). The molecular weights and anti-tubulin reactivity are similar to those of the S1 and S2 proteins of spirochetes (11), with the higher-molecular-weight spirochete protein now known to be a heat shock protein.

(ii) Other bacterial taxa. Tubules or fibers are also seen in *Arthromitus* spp. and in other unidentified termite gut bacteria (Fig. 7). In *Arthromitus* spp. they occur as endospore appendages (88) (Fig. 7A). The actinobacterium *Frankia* strain HFPCcI3, symbiotic with *Casuarina cunninghamiana*, possesses tubules which are well preserved by freeze-substitution (Fig. 7E) but are larger than those of eukaryotes (71). A tubule seen in group D streptococcal L-forms which extended beyond the cell is probably cell wall material (23). Cores, which are tubules postulated to be of membranous origin, were also seen in group D streptococci (22).

Archaebacteria

Halobacteria. Although no tubules were observed in thin section, whole-cell extracts of the archaebacterium *Halobacterium halobium* reacted positively with an anti-tubulin antibody to a 55-kDa band. Growth was inhibited by tubulin-binding drugs: vincristine, podophyllotoxin, and nocodazole (117). Probing of genomic digests of *H. halobium* with a labeled plasmid containing the yeast α-tubulin gene gave a faint positive response indicating potential sequence homology between *Saccharomyces* tubulin and genes of *H. halobium* (117).

DISCUSSION

Cytoplasmic tubules, fibers, and tubulin-like proteins have been reported to occur in over 50 taxa of prokaryotes (Table 1). Most entries in Table 1 are restricted to descriptive morphology; lacking definitive molecular biological data, none of the reports is adequate to conclude the presence of tubulin in any prokaryote. However, the report of a conserved tubulin motif in a GTP-binding protein, the *ftsZ* gene product of *E. coli* and other bacteria, suggests that proteins ancestral to tubulins may have originated in bacteria prior to the evolution of eukaryotes. Other reports, including those concerning *Azotobacter* and *Halobacterium* strains, also seem promising and warrant further investigation.

The term "microtubule" should be reserved for tubulin-containing structures. No report of bacterial "microtubules" conforms to the standard biochemical, morphological, and sequence definition used by cell biologists who study eukaryotes. Prokaryotic tubules must be assumed nonhomologous to eukaryotic microtubules until sequence homologies are confirmed. Because they seem to differ greatly even from each other, we conclude that most prokaryotic hollow structures are probably not tubulin microtubules. The evolutionary homology of eukaryotic microtubules is well established now that sequence data are available for more than 50 representative organisms (78, 104). Microtubules develop from MTOCs in eukaryotic cells. Some of these MTOCs, like the [9(3)+0] kinetosomes, are highly organized and evolutionarily conserved, whereas others, like those of fungi (41) and certain protists (123) (Fig. 2), are much more amorphous, at least at certain stages in cell development. If an evolutionary relation-

ship exists between eukaryotic microtubules and their MTOCs and any of the various cytoplasmic prokaryotic tubules (or proteins such as FtsZ), it remains to be demonstrated.

Certain drugs that specifically inhibit tubulin polymerization or depolymerization can aid in identifying microtubules. However, drug insensitivity results can never definitively exclude the possibility that prokaryotic tubules are homologous to microtubules. Tubulin inhibitor drugs are taxon dependent; e.g., colchicine-insensitive tubulin is common in cells from protists or fungi, and plant tubulin is far more sensitive to oryzalin and benomyl than is mammalian brain tubulin. Drug resistance can occur as a result of single amino acid substitutions (63, 64). Some of the tubulin-active drugs can be metabolized by bacteria, showing that inhibitors may not necessarily reach their potential sites of action. Any claim about "bacterial tubulin" based on drug inhibition requires direct methods of detection. Similarly, attempts to identify tubulin in prokaryotes by use of antibodies have been misleading (98). Some of the previous reports of antibodies to tubulin reacting with prokaryotes are attributable to hsp65. Sequence analyses would be necessary to prove that cytoplasmic tubules of prokaryotes are indeed homologous to eukaryotic microtubules.

The regularity with which tubules in cyanobacteria, spirochetes, and other bacteria have been observed suggests that they should not be dismissed as artifacts. Cyanobacterial tubules in particular and bacterial tubules in general exhibit varied morphology. Whatever the composition of prokaryotic tubules, their presence poses questions concerning their function. Some, such as those of *Vibrio psychroerythrus*, traverse the septum during cell division. Many of the tubules and fibers exist in wall-less bacteria, larger cells, or elongate bacteria and are oriented longitudinally. Such structures may be involved in a variety of functions such as intracellular transport, genome segregation, gliding or other movement, and cytoskeletal functions including generation of cell asymmetry during morphogenesis. These questions will remain unanswered until biochemical characterization permits physiological and genetic analysis.

The origin of tubulin and microtubules is unknown. No tubulin has yet been definitively detected in any bacterium. However, it would be premature to conclude that they are absent in prokaryotes. The presence of cytoplasmic tubules in selected genera of azotobacteria, cyanobacteria, enteric bacteria, mycoplasms, spirochetes, and possibly archaebacteria is evident; whether or not any are homologous to eukaryotic tubulin microtubules has yet to be established with certainty.

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ADDENDUM IN PROOF

Bramhill and Thompson (D. Bramhill and C. M. Thompson, Proc. Natl. Acad. Sci. USA **91**:5813–5817, 1994) report that the purified FtsZ GTPase from *E. coli* forms tubules (14 to 20 nm, outer diameter) that are estimated to be composed of 12 or 13 protofilaments in parallel alignment. The rate of FtsZ polymerization is approximately 100 times more rapid than that of tubulin. This property together with those previously described makes FtsZ the best-known candidate in prokaryotes for the evolutionary precursor of eukaryotic tubulin protein.

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